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Contact and Artificial Soil Tests Using Earthworms to Evaluate the Impact Wastes in Soil

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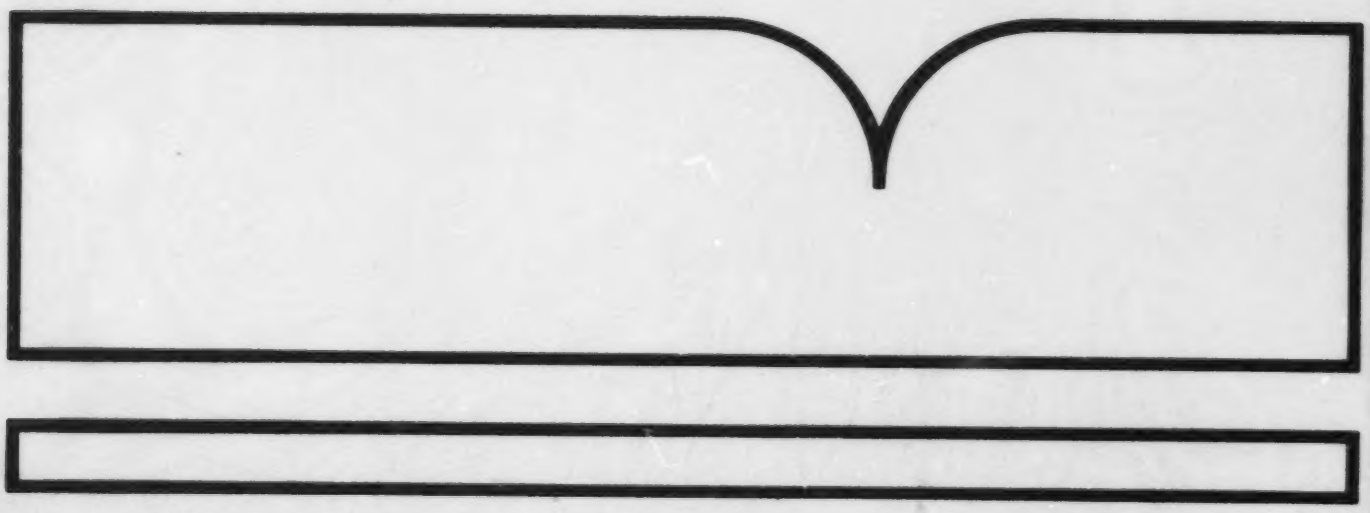

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16. ABSTRACT

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These studies have shown that 1) earthworms can be used to distinguish among a wide variety of chemicals with a high degree of accuracy, 2) earthworms are a suitable biomonitoring tool to measure the impact of chemicals in wastes added to soils, 3) the contact & artificial soils tests can measure the biological impact of chemicals applied to soils, 4) earthworms can be used to differentiate the impact of chemical groups and specific chemicals, and 5) the contact & artificial soil tests appear to identify the same relative toxicity of chemicals as rat toxicity data.

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Edward F. Neuhauser,[1] *Raymond C. Loehr,*[2] *and Michael R. Malecki*[1]

Contact and Artificial Soil Tests Using Earthworms to Evaluate the Impact of Wastes in Soil

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ABSTRACT: This study was designed to evaluate two methods using earthworms that can be used to estimate the biological impact of organic and inorganic compounds that may be in wastes applied to land for treatment and disposal. The two methods were the contact test and the artificial soil test. The contact test is a 48-h test using an adult worm, a small glass vial, and filter paper to which the test chemical or waste is applied. The test is designed to provide close contact between the worm and a chemical, similar to the situation in soils. The method provides a rapid estimate of the relative toxicity of chemicals and industrial wastes. The artificial soil test uses a mixture of sand, kaolin, peat, and calcium carbonate as a representative soil. Different concentrations of the test material are added to the artificial soil, adult worms are added, and worm survival is evaluated after two weeks.

These studies have shown that (1) earthworms can be used to distinguish among a wide variety of chemicals with a high degree of accuracy, (2) earthworms are a suitable biomonitoring tool to measure the impact of chemicals in wastes added to soils, (3) the contact and artificial soil tests can measure the biological impact of chemicals applied to soils, (4) earthworms can be used to differentiate the impact of chemical groups and specific chemicals, and (5) the contact and artificial soil tests appear to identify the same relative toxicity of chemicals as rat toxicity data.

KEY WORDS: land treatment, contact test, artificial soil test, industrial wastes, hazardous wastes, earthworms, biological testing, heavy metals, organic chemicals

Land application has been used successfully to treat many industrial wastes. However, laboratory and field studies of this treatment method rarely

[1]Research associate and research support specialist, respectively, Department of Agricultural Engineering, Cornell University, Ithaca, NY 14853.

[2]Professor of civil engineering, University of Texas, Austin, TX 78712; formerly, professor of agricultural engineering and environmental engineering, Cornell University, Ithaca, NY 14853.

consider the impact of potentially hazardous waste materials on the soil ecosystem. This paper evaluates methods that measure the impact of waste materials on earthworms, an important component of the soil biota. Previous work with this approach was reported at an earlier ASTM symposium [*1*].

The complexity of soil fauna is a complicating factor when biological assays are used to evaluate the impact of wastes on the soil ecosystem. Soil microorganisms and soil arthropods are very diverse, difficult to identify, and difficult to use in biological testing procedures. Earthworms, a major biological component of soils, are relatively easy to obtain in large numbers and biomass and can be a suitable soil test organism.

This paper discusses the applicability of two methods originally developed by the European Economic Community (EEC) [*2*] to estimate the biological impact of wastes applied to soils and wastes leaking from landfills and impoundments. These methods are designed to evaluate the short-term impact of materials on earthworm survival through skin absorption (contact test) and the longer term impact on earthworm survival through skin absorption and ingestion (artificial soil test).

Procedures

Earthworms

Eisenia fetida (Savigny) was the earthworm species used in these test methods. Large numbers of this species of known age and size can be obtained easily for testing. *Eisenia fetida* occurs in specific habitats in temperate climates; considerable information has accumulated concerning its growth [*3*], reproduction [*4*], physical requirements [*5*], use in waste management systems [*6*], and response to metals [*7,8*]. This body of information and the ease of using this species under laboratory conditions has made it appropriate to use *E. fetida* in biological testing methods.

The worms used in these experiments were obtained from stock cultures in the laboratory of the authors. The cultures are maintained at room temperature (21 to 24°C), with cocoons being harvested every two weeks. Aerobically digested domestic sludge was used as the maintenance organic food substrate for the worms.

Chemicals

Both organic and inorganic chemicals were used to evaluate the potential impact of wastes applied to soils. The water soluble salts (acetate, chloride, nitrate and sulfate) of five metals (cadmium, copper, nickel, lead, and zinc) were selected on the basis of their potential toxicity and presence in municipal and industrial wastes that may be applied to soils. All of the metal salts used

were Fisher-certified chemicals. The organic chemicals used in these studies were organic priority pollutants [*9*] and were at least 98% pure.

Contact Test

The contact test procedures described by the EEC [*2*] were followed. In this test, glass vials are lined with filter paper (Whatman No. 1) to which the chemical is added. The chemical is distributed over the surface of the paper, one worm is added to each vial, and the vials are incubated at 20°C. The worm is in contact with the chemical and can absorb the chemical through its skin. After incubation for a defined time period (two days at 20°C), worm survival is determined. A range of chemical concentrations is evaluated and a large number of replicates are used for each concentration. The results are evaluated to determine a median lethal concentration (LC_{50}) value for each chemical.

Glass vials [9 mL (3 oz)] were lined with 12 by 6.5 cm of filter paper to give an area of 80 cm^2 (Fig. 1). Fresh aqueous metal salt solutions were made for each series of tests.

The testing of organic chemicals used distilled water as the solvent if possible. If the test chemical was not soluble in water, other solvents (acetone or chloroform) were used. The organic solvent used to add the chemical should volatilize readily and be evaporated from the vial before the 1 mL of distilled water and the worm are added to the vial. The loss of organic solvents was evaluated and did occur in all cases before the worms were added. In addition, experimentation determined that none of the chemicals added to the vials with the organic solvents volatilized with the organic solvent. The 1 mL of water was added to provide enough moisture for earthworm survival. One adult earthworm (300 to 500 mg) was added per vial and the vials were kept on their side at 20°C in a darkened incubator. Only one worm is added per vial because the death of one worm in the vial could adversely affect the well-being of any other worms present.

After 48 h, the viability of the earthworms was determined. Earthworms were considered dead if they did not respond to a gentle mechanical stimulus.

FIG. 1—*Cutaway view of glass vial lined with filter paper (12 cm by 6.7 cm, or 80 cm^2 per vial) used for the two-day contact test at 20° C.*

The number of dead worms was related to the concentration of the chemical to which the worms were exposed.

The LC_{50} value for each chemical tested was obtained by first using a series of screening tests to determine the range of chemical concentrations that had an adverse impact. This permitted the use of a narrow, more definitive range to determine the more specific LC_{50} value. The range finding tests included concentrations that resulted in mortalities from 0 to 100%. This was accomplished by using chemical concentrations that varied over several logs.

At least five concentrations were evaluated in the definitive test, with ten or more replicates included for each concentration tested. Controls, which included vials that contained everything except the test chemical, were included in each series of experiments.

The LC_{50} value for each chemical tested was calculated using the method of Litchfield and Wilcoxon [*10*]. In the contact test, the LC_{50} values are reported in terms of micrograms of test chemical per square centimetre of filter paper.

Artificial Soil Test

The EEC tentative artificial soil test method [*2*] was followed exactly. This test uses a medium which consists by weight of 10% finely ground sphagnum peat, 20% colloidal kaolin clay, 69% fine sand, and 1% pulverized calcium carbonate. The final pH was adjusted to 6.0 ± 0.5. The water content was raised to 35% of the dry weight of the four components. The test chemical was dissolved in water and then added to the artificial soil.

If the test chemical was not water soluble, it was mixed with a small volume of suitable organic solvent (acetone or chloroform) and then mixed with the artificial soil. Tests were run to determine the amount of time needed to completely evaporate the organic solvent from the artificial soil. After the organic solvent evaporated, the artificial soil was brought to a moisture content of 35%. Controls, which consisted of all components except the test chemical, were included in each set of experiments.

Ten adult *E. fetida* (300 to 500 mg) were added to 400 g (dry weight) of the test mixture in round glass dishes 6.5 cm high and 12.5 cm in diameter (Fig. 2). The dishes were placed in an incubator at 20°C and mortality was assessed after two weeks. Earthworms were considered dead when they did not respond to a gentle mechanical stimulus.

Concentrations causing mortalities of 0 to 100% were determined by using range-finding tests that included chemical concentrations that varied over several logs. After the range of chemicals had been narrowed, a definitive test was performed. This test had at least five chemical concentrations, with four repetitions for each test concentration and four control dishes, all containing ten worms per dish.

The LC_{50} value for each chemical was calculated by using the method of

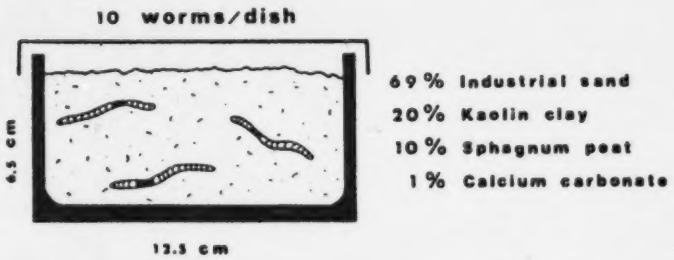

FIG. 2—*Cutaway view of glass dish filled with artificial soil (400 g) used for the 14-day artificial soil test at 20° C.*

Litchfield and Wilcoxon [*10*] and is reported in terms of milligram chemical per kilogram dry weight of artificial soil.

Results

Metals

Four salts of each metal were used in the contact test to determine if the metal salts exhibited different toxicities (Table 1). Statistical analyses determined that the four salts of each metal (acetate, chloride, nitrate, and sulfate) had similar LC_{50} values. The different water-soluble salts of each metal did not show any difference in toxicity to the worms. Of the metals tested, copper was the most toxic, followed by zinc, nickel, cadmium, and lead in order of decreasing toxicity.

The artificial soil test requires more work than the contact test. Because none of the metal salts showed any differences, only one salt (nitrate) was

TABLE 1—*The earthworm contact test LC_{50} values for the metals tested.*

Metal	Metal Salt LC_{50}, $\mu g/cm^2$ (95% CI)[a]			
	Acetate	Chloride	Nitrate	Sulfate
Cadmium	20 (16-25)[A]	18 (12-25)[A]	24 (12-45)[A]	26 (17-39)[A]
Copper	6.7 (5.3-8.5)[B]	4.9 (4.0-5.9)[B]	7.4 (3.8-14.4)[B]	6.3 (4.6-8.6)[B]
Lead	50 (30-83)[C]	NT[c]	64 (45-92)[C]	NT[c]
Nickel	17 (13-22)[D]	17 (12-24)[D]	25 (19-34)[D]	20 16-25)[D]
Zinc	13 (10-16)[E]	12 (6-24)[E]	10 (8-12)[E]	13 (7-26)[E]

[a]CI = confidence interval.
[A]Means with a common superscript symbol are not significantly different, $P < 0.05$.
[c]Not tested.

used in the artificial soil test. The LC_{50} values obtained for the nitrate salts of the five metals tested in the artificial soil test are noted in Table 2.

The artificial soil test results also indicated that copper, nickel, and zinc were the most toxic metals, with no statistical difference among them. Cadmium was the next most toxic metal tested, followed by lead.

Organics

The results of the 44 organics tested with the earthworm contact test are summarized in Table 3. The phenols were the most toxic chemical group tested, followed closely by the amines. The major remaining chemical groups, in order of toxicity from most toxic to least toxic, were substituted aromatics, halogenated aliphatics, polycyclic aromatic hydrocarbons, and phthalates. The individual contact test results for the 44 chemicals are given in Table 4.

The individual LC_{50} values for one of the chemical groups studied, the substituted aromatics, are summarized in Table 5. There was no statistical dif-

TABLE 2—*Artificial soil test LC_{50} values for the nitrate salts of the metals tested.*

Metal	Artificial Soil Test LC_{50}, mg/kg (95% CI)
Cadmium	1843 (1660-2045)
Copper	643 (549-753)[a]
Lead	5941 (5292-6670)
Nickel	757 (661-867)[a]
Zinc	662 (574-674)[a]

[a]Means footnoted are not significantly different, $P < 0.5$.

TABLE 3—*Summary of means and ranges for the six chemical groups in the contact test and the means and ranges for the rat oral LD_{50} values for the same chemical groups.*

Chemical Group[a]	Worm LC_{50}, $\mu g/cm^2$ (range)	Rat Oral LD_{50}, mg/kg (range)
Phenols ($n = 9$)	2.9 (0.6-5.9)	1 000 (30-3 200)
Amines ($n = 3$)	5.0 (2.0-11.0)	740 (70-1 650)
Substituted aromatics ($n = 9$)	41 (14-98)	2 100 (250-5 000)
Halogenated aliphatics ($n = 14$)	93 (10-304)	2 300 (75-10 000)
Polycyclic aromatic hydrocarbons ($n = 4$)	1 470 (49-4 670)	4 560 (1 780-10 000)
Phthalates ($n = 5$)	1 760 (550-3 140)	20 225 (6 900-31 000)

[a]n = number of chemicals tested with the contact test.

TABLE 4—*Earthworm LC_{50} contact test values, amounts of chemicals used, water solubilities, and vapor pressures for the 44 chemicals tested with the contact test.*

Chemical	Contact Test LC_{50}, µg/cm² (95% CI)[a]	Chemical[b] Solubility at 20°C[c] µg per Vial	µg/mL	Vapor Pressure, mm[c]
Phenols				
2,4-Dinitrophenol	0.6 (0.5-0.7)[e,d]	48	5 620	N
4-Nitrophenol	0.7 (0.6-0.8)[e]	56	16 000	2.2
2-Chlorophenol	2.2 (1.9-2.4)[f]	176	28 500	40
2,4-Dimethylphenol	2.2 (1.7-2.8)[f]	176	4 200	0.06
Pentachlorophenol[f]	2.4 (1.8-3.0)[f]	192	10^{-3}	14
2,4-Dichlorophenol	2.5 (2.3-2.8)[f]	200	4 600	0.12
2,4,6-Trichlorophenol	5.0 (4.1-6.1)[g]	400	800	1.0
Phenol	5.0 (4.2-5.8)[f]	400	82 000	0.2
2-Nitrophenol	5.9 (5.5-6.3)[g]	472	2 100	1.0
Amines				
Chloroacetamide	2.0 (1.9-2.1)[e]	160	>10 000	NF
Diphenyl nitrosamine[f]	2.4 (2.2-2.6)[f]	192	<2.4	NF
Di-*n*-propyl nitrosamine	11.0 (10.0-12.0)[g]	880	9 900	NF
Substituted aromatics				
Carbaryl[f]	14 (10-19)[e]	1 120	40	0.15
Nitrobenzene	16 (15-17)[e]	1 280	1 900	0.15
1,2-Dichlorobenzene[f]	21 (19-23)[f]	1 680	145	1.0
1,2,4-Trichlorobenzene[f]	27 (24-32)[g]	2 160	19	0.42
Chlorobenzene[f]	29 (22-38)[g]	2 320	500	8.8
Ethylbenzene[f]	47 (42-53)[h]	3 760	150	7.0
Toluene[f]	75 (68-83)[h]	6 000	5.0	10
Benzene[f]	98 (78-123)[h]	7 840	1 780	76
Hexachlorobenzene[f]	nontoxic up to 1 000[e]	80 000	0.1	10^{-5}
Halogenated aliphatics				
Hexachlorobutadiene[f]	10 (9-11)[e]	800	2.0	0.15
Tetrachloroethane	14 (11-17)[e,f]	1 120	2 900	5
Hexachloroethane[f]	19 (16-23)[f,i]	1 520	50	0.4
Bis(2-chloroethyl)ether	19 (18-20)[f]	1 520	10 200	0.7
2-Chloroethyl vinyl ether	33 (31-36)[h]	2 640	15 000	26
1,1,2-Trichloroethane	42 (35-49)[h]	3 360	4 500	19
1,2-Dichloroethane	60 (54-68)[h]	4 800	8 700	61
1,2-Dichloropropane[f]	64 (59-70)[h]	5 120	2 700	42
1,1,1-Trichloroethane[f]	83 (76-91)[g]	6 640	4 400	100
Trichloroethene[f]	105 (91-122)[e,e]	8 400	1 100	60
Trichloromethane[f]	111 (103-122)[e,e]	8 880	8 000	160
Tetrachloromethane[f]	160 (142-179)	12 800	800	90
1,2-*trans*-Dichloroethene[f]	286 (264-308)[i,i]	22 880	800	200
Dichloromethane[f]	304 (258-358)[i,i]	24 320	20 000	349
Polycyclic aromatic hydrocarbons				
Acenaphthene[f]	49 (34-69)[e]	3 920	3.4	10^{-3}
Fluorene[f]	171 (128-224)[f]	13 680	1.8	10^{-3}
Fluoranthene[f]	2 160 (1 550-3 000)[g]	172 800	0.2	10^{-5}
Naphthalene[f]	4 670 (2 730-7 980)[h]	373 600	3.4	0.5

TABLE 4—*Continued.*

Chemical	Contact Test LC_{50}, $\mu g/cm^2$ (95% CI)[a]	Chemical[b] Solubility at 20°C[c] μg per Vial	$\mu g/mL$	Vapor Pressure, mm[c]
Phthalates				
Dimethyl phthalate	550 (440-690)[e]	44 000	5 000 000	<0.01
Diethyl phthalate	850 (660-1 090)[f]	68 000	1 000 000	14
n-Butyl phthalate[d]	1 360 (1 050-1 750)[g]	108 800	13 000	0.1
Dioctyl phthalate[d]	3 140 (2 270-4 330)[h]	251 200	3 000	1.2
Bis(2-ethylhexyl)phthalate[d]	nontoxic up to 25 000	2×10^6	400	<0.01

[a]Numbers in parentheses are the 95% confidence intervals (CI).
[b]Indicates the total amount of chemical present per vial at the LC_{50} concentration.
[c]Data from Ref 9.
[d]Means with a common superscript symbol are not significantly different ($P < 0.05$) within a chemical group.
[e]NF—not found.
[f]The amount of chemical in the vial at the LC_{50} concentration exceeds the solubility of the chemical in 1 mL of water.

TABLE 5—*Earthworm LC_{50} contact test values for the substituted aromatics tested.*

Chemical	LC_{50}, $\mu g/cm^2$ (95% CI)
Carbaryl	14 (10-19)[a,e]
Nitrobenzene	16 (15-17)[a]
1,2-Dichlorobenzene	21 (19-23)
1,2,4-Trichlorobenzene	27 (24-32)[f]
Chlorobenzene	29 (22-38)[f]
Ethylbenzene	47 (42-53)
Toluene	75 (68-83)[g]
Benzene	98 (78-123)[g]
Hexachlorobenzene	nontoxic up to 1 000 $\mu g/cm^2$

[a]Means with a common superscript symbol are not statistically different, $P < 0.05$.

ference in toxicity between carbaryl and nitrobenzene, the most toxic substituted aromatics tested. The chlorine-substituted aromatics were all more toxic than the alkyl-substituted aromatics. Hexachlorobenzene, perhaps due to its extremely low solubility, 0.1 $\mu g/mL$, was the least toxic of all the substituted aromatics tested. The two-day contact test may not have permitted enough time for the worms to be affected by the hexachlorobenzene.

The large amount of effort involved in conducting the artificial soil tests limited the number of chemicals that could be evaluated. Table 6 lists the

TABLE 6—*Impact of chemicals in the artificial soil test and contact test.*

Chemical	Artificial Soil Test LC_{50}, mg/kg (95% CI)	Contact Test LC_{50}, $\mu g/cm^2$ (95% CI)
Chloroacetamide	26 (22-31)	2.0 (1.9-2.1)
4-Nitrophenol	38 (32-45)	0.7 (0.6-0.8)
2,4,6-Trichlorophenol	58 (54-62)	5.0 (4.1-6.1)
Carbaryl	106 (81-138)	14 (10-19)
Diphenyl nitrosamine	151 (136-167)	2.4 (2.2-2.6)
Fluorene	173 (150-201)	171 (128-224)
Phenol	401 (347-463)	5.0 (4.2-5.8)
2-Chloroethyl vinyl ether	740 (570-970)	33 (31-36)
Dimethyl phthalate	3 160 (2 450-3 920)	550 (440-690)
1,2-Dichloropropane	4 240 (3 830-4 680)	64 (59-70)

LC_{50} values found for the organics tested with the artificial soil test in order of decreasing toxicity. The contact test LC_{50} values for the organics tested with the artificial soil test are included for comparative purposes. In general, the chemicals that were the most toxic to the worms in the contact test were the most toxic in the artificial soil test.

Discussion

The development of any bioassay or toxicity index requires that the data obtained with the new methods be compared to existing toxicity data. One of the most readily available toxicity data bases is rat oral lethal dose for 50% (LD_{50}) data. Table 3 lists the mean earthworm LC_{50} values and ranges and the mean rat oral LD_{50} values and range for the six chemical classes tested. The two chemical groups that were the most toxic to the earthworms, phenols and amines, were also the most toxic to the rat. The third most toxic group to the earthworms, substituted aromatics, was also the third most toxic chemical group to the rat. The other three chemical groups, halogenated aliphatics, polycyclic aromatic hydrocarbons and phthalates, were less toxic to the earthworms and the rats but had the same relative toxicity. This comparison suggests that chemicals toxic to the earthworms have the same relative toxicity to rats and vice versa.

Comparable data for specific organic chemicals, in terms of earthworm LC_{50} and rat LD_{50} values, are presented in Table 7. This comparison also shows the same relative toxicity pattern. The substituted aromatics that were the most toxic to the rat also were the most toxic to the worms. Carbaryl, nitrobenzene, and 1,2-dichlorobenzene were the three most toxic substituted aromatics and toluene, benzene, and hexachlorobenzene were the three least toxic substituted aromatics tested.

The LC_{50} data from the artificial soil test also were compared to the rat

TABLE 7—*Earthworm LC_{50} contact test values and rat oral LD_{50} values for the substituted aromatics tested.*

Chemical	Contact Test LC_{50}, μg/cm²	Rat Oral LD_{50}, mg/kg
Carbaryl	14[a,e]	250
Nitrobenzene	16[a]	640
1,2-Dichlorobenzene	21	500
1,2,4-Trichlorobenzene	27[f]	756
Chlorobenzene	29[f]	2 910
Ethylbenzene	47	3 500
Toluene	75[f]	5 000
Benzene	98[f]	3 800
Hexachlorobenzene	nontoxic up to 1 000 μg/cm²	10 000

[a]Means with a common superscript symbol are not significantly different, $P < 0.05$.

LD_{50} data (Table 8). Again, in general, the chemicals that were most toxic to the worms also were the most toxic to the rat.

A comparison of the relevant factors associated with the contact and artificial soil tests (Table 9) identifies the advantages and disadvantages of both methods. The contact test is more rapid and less expensive to run than the artificial soil test. The artificial soil test may be more representative of what really happens to earthworms in soil ecosystems since this test permits both skin contact and ingestion of the test chemical. Neither test lasts long enough to evaluate growth and reproductive effects.

The comparisons of relative order of toxicity (Tables 6, 7, and 8) indicated that nitro-substituted compounds were generally more toxic than chloro-

TABLE 8—*Impact of the chemicals in the artificial test and rat oral LD_{50} values.*

Chemical	Artificial Soil Test LC_{50}, mg/kg	Rat oral LD_{50}, mg/kg
Chloroacetamide	26	70
4-Nitrophenol	38	350
2,4,6-Trichlorophenol	58	820
Carbaryl	106	250
N-Nitrosodiphenylamine	151	1 650
Fluorene	173	NF[a]
Phenol	401	414
2-Chloroethyl vinyl ether	740	250
Dimethyl phthalate	3 160	6 900
1,2-Dichloropropane	4 240	1 900

[a]Not found.

TABLE 9—*Comparison of the parameters involved with the contact and artificial soil tests.*

Parameter	Contact Test	Artificial Soil Test
Time	short—2 days	long—14 days
Cost	lower	higher
Mode of impact	topical	topical and ingestion
Usefulness	for compounds that pass through skin membranes	for low-volatility compounds
Complexity of test	fewer components to procedure	more components to procedure

substituted compounds, which in turn were more toxic than alkyl-substituted compounds. All of the substituted compounds were more toxic than the parent unsubstituted compound.

The contact test and the artificial soil test are biological assay methods that can be used to estimate the biological impact of wastes and chemicals added to the soil. Both are relatively short-term methods: the contact test takes two days and the artificial soil test takes 14 days. The length of a biological assay can affect the toxicity of a chemical. If the chemical takes a long time to affect the biological system of a worm, the contact and artificial soil tests may not identify the true toxicity. An example could be cadmium, which in both the contact and artificial soil tests was not as toxic as copper, zinc, or nickel. However, in our other studies [*7*], in which the same five metals were analyzed for their effect on growth and reproduction, cadmium was the most toxic of the five metals tested.

Summary

The contact test and artificial soil tests are designed to screen the biological impact of potentially toxic chemicals in wastes applied to soils. Four salts (acetate, chloride, nitrate and sulfate) of five metals (cadmium, copper, nickel, lead, and zinc) were evaluated by using the contact test. The different water-soluble salts of each metal did not indicate any difference in toxicity to the worms. The order of toxicity for the metals in the contact test, from most toxic to least toxic, was Cu · Zn · Ni ≃ Cd · Pb. The order of toxicity for the metals in the artificial soil test, from most toxic to least toxic, was Cu ≃ Zn ≃ Ni · Cd · Pb.

A number of organic chemicals were tested for potential toxicity by using both the contact test and artificial soil test. The results were compared to available information on the rat oral LD_{50} values for the same compounds. The comparison indicated similarity between the relative toxicity of the chem-

icals to the worm and the rat. The chemicals that were most toxic to the worm were most toxic to the rat and those that were least toxic to the worm were least toxic to the rat.

The same relative toxicity was apparent for the six major chemical groups that were tested. The two most toxic chemical groups to the worms, phenols and amines, were also the most toxic to the rat, and the two least toxic chemical groups to the worms, polycyclic aromatic hydrocarbons and phthalates, were the two least toxic chemical groups to the rat.

The earthworm contact and artificial soil tests are useful in predicting the potential biological impact of organics and inorganics in wastes that may be applied to land for treatment and disposal.

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References

[1] Neuhauser, E. F., Malecki, M. R., and Loehr, R. C., in *Hazardous and Industrial Solid Waste Testing (Second Conference). ASTM STP 805*, American Society for Testing and Materials, Philadelphia, 1983, pp. 313-320.

[2] *EEC Guidelines for the Testing of Chemicals*, "The Contact and Artificial Soil Tests," EEC 79/831, Rev. 3., European Economic Community, Brussels, Belgium, 1982.

[3] Neuhauser, E. F., Hartenstein, R., and Kaplan, D. L., *Oikos*, Vol. 35, 1980, pp. 93-98.

[4] Hartenstein, R., Neuhauser, E. F., and Kaplan, D. L., *Oecologia*, Vol. 43, 1979, pp. 329-340.

[5] Kaplan, D. L., Hartenstein, R., Neuhauser, E. F., and Malecki, M. R., *Soil Biology and Biochemistry*, Vol. 12, 1980, pp. 347-352.

[6] Neuhauser, E. F., Kaplan, D. L., Malecki, M. R., and Hartenstein, R., *Agricultural Wastes*, Vol. 2, 1980, pp. 43-60.

[7] Malecki, M. R., Neuhauser, E. F., and Loehr, R. C., *Pedobiologia*, Vol. 24, 1982, pp. 129-137.

[8] Neuhauser, E. F., Malecki, M. R., and Loehr, R. C., *Proceedings*, 37th Purdue Industrial Waste Conference, Ann Arbor Science Publishers, Ann Arbor, MI, 1983, pp. 253-258.

[9] "Water-Related Environmental Fate of 129 Priority Pollutants," EPA-440/4-79-029, U.S. Environmental Protection Agency, Washington, DC, 1979.

[10] Litchfield, J. T. and Wilcoxon, F., *Journal of Pharmacology and Experimental Therapeutics*, Vol. 96, 1949, pp. 99-113.